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Atypical/malignant urothelial cells in routine urinary sediment: Worth knowing and reporting

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ABSTRACT

Background: Urinary cytology (Ucytol), which is performed in pathology laboratories on fixed and stained samples, represents the gold standard for the identification of atypical/malignant urothelial cells (A/MUC) due to urothelial carcinoma. In this paper we describe three patients in whom A/MUC, due to a bladder carcinoma, were identified with conventional urine sediment (Used) examination on unfixed and unstained samples.
Methods: Included are urine samples prepared with conventional and standardized techniques as currently used in general clinical laboratories. Samples were examined with phase contrast microscopy. A/MUC were identified according to the criteria currently used for Ucytol.
Results: A/MUC (i.e., cells with unusual and pleomorphic size and shape, increased nuclear/cytoplasmic ratio, increased number of nuclei, irregular nuclear borders and irregular chromatin patterns, either isolated or in clusters) were identified in the urine of three patients, all of whom were found to have bladder carcinoma by cystoscopy.
Conclusions: At variance with the common and widespread view, A/MUC can also be identified with conventional Used examination, even though Ucytol still represents the gold standard method.

1. Introduction

The search in urine of atypical/malignant urothelial cells (A/MUC) [1–5] represents, together with cystoscopy, the gold standard approach for the diagnosis of bladder carcinoma and its recurrence at follow-up [6]. This investigation is based on cytological techniques which are carried out in pathology laboratories by experienced personnel [6].

Urinary sediment (Used) examination is an integral part of routine urinalysis, which allows the identification of several categories of particles (i.e., cells, lipids, casts, crystals, microorganisms and contaminants), most of which have clinical implications [7,8]. UCs, either superficial or deep, are well known particles, whose presence in the Used is considered a marker of damage of the multilayered uroepithelium which lines the urinary excretory system from the renal calyces to the bladder in female and to the proximal urethra in male [7,8]. However, the current view is that A/MUC cannot be reliably identified by routine Used examination.

At variance with this widespread opinion, few reports show that A/MUC can also be identified in unstained urine samples, as commonly examined in general clinical laboratories [9,10].

The aim of this paper is to add further support to this view by describing three patients with bladder carcinoma whose A/MUC were identified by conventional Used examination.

2. Materials and methods

2.1. Urine microscopy

For all three patients the urine samples were collected and prepared according to a standardized method described in detail elsewhere [11]. Briefly, a 10 mL aliquot of the second urine of the morning, produced over a 2-hour interval, was centrifuged at 400 *g* (=2000 rpm) for 10 min in a 10 mL graduated glass tube with a conical bottom; then 9.5 mL of the supernatant urine was removed by a water pump, and the sediment was gently but thoroughly resuspended with a Pasteur pipette in the remaining 0.5 mL of urine; then a 50 μL aliquot of resuspended sediment was transferred by a precision pipette to a glass slide, which was covered with a 24 × 32 mm coverslip. The urine samples of patients 1 and 3 were analyzed with a phase contrast Leitz Dialux 20 microscope (Leica Microsystems, GmbH, Wetzlar, Germany) at institution 1 (Clinical and Research Laboratory on Urinary Sediment, U.O. di Nefrologia, Ospedale Policlinico, Milan, Italy); the sample of patient 2 was examined with a phase contrast Leica DMLS microscope, at institution 2 (Clinical Laboratory of Saint Jean de Dieu Hospital, Tanguiéta,

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Bénin Republic, West Africa). In both institutions the samples were examined within 3 h of urine collection.

The microscopists of the urine were two of the authors (GG at institution 1 and GBF at institution 2), both with an expertise in Used [7,12]. The samples were first screened at low magnification (160× at institution 1; 100× at institution 2), then at 400× at both institutions. Cells were quantified as average number/high power field (HPF).

2.2. Morphological criteria for UC atypia/malignancy

UCs were considered atypical/malignant when they had one or more of the following features [1–5]: unusual cell size and shape, enlarged nucleus (= high N/C ratio), irregular nuclear borders, irregular chromatin pattern, increased number of nuclei, and tendency to aggregate in clusters of variable size and shape.

The measurement of cells and nuclei of A/MUC was performed by a digital micrometric system mounted on the digital camera (Leica DC 300 F) connected with the microscope.

2.3. Histological evaluation of the urothelial lesions

The pathology reports of the urothelial lesions of patients 1 and 3 were recovered from the pathology archives. The tissue specimens for the confirmation of bladder carcinoma were reviewed by one of us (PF).

3. Results

3.1. Patient 1

On July 10th 2007, a 55-year-old Eritrean man was admitted to Policlinico hospital of Milan for gross hematuria, abdominal pain, and fever. Serum creatinine was 398 μmol/L (n.v. 44–88). Ultrasounds of the abdomen showed a solid mass in the bladder and in the right kidney, and left hydronephrosis.

Urinalysis by dipstick showed: pH: 5.0, S.G.: 1.020, albumin: +, hemoglobin: ++++, leukocyte esterase: negative, and nitrites: absent. Used examination revealed: >100 isomorphic red blood cells (RBCs)/HPF, >1 normal UC/HPF both superficial and deep, and >1 A/MUC/HPF, both isolated and in clusters. The A/MUC had a spindle shape and a central or a peripheral nucleus, which contained enlarged nucleoli and often irregular chromatin patterns (Fig. 1A–C). The cell major diameter, measured on 18 cells, ranged from 12.2 to 76.1 μm (mean ± SD: 38.2 ± 21.6), and that of nuclei ranged from 7.4 to 13.6 μm (10.0 ± 1.6).

After a cystoscopy which confirmed the presence of a solid mass, on July 30th the patient underwent a radical cystectomy and ureterocutaneostomy. The histological examination of the lesion showed a urothelial papillary carcinoma of low-grade malignancy of the left side wall of the bladder (Fig. 1D), an outbreak of urothelial carcinoma in situ of the bladder mucosa at the dome and a focal urothelial papillary carcinoma of high-grade malignancy in the intramural portion of the ureter infiltrating the lamina propria.

3.2. Patient 2

On February 7th 2008, a 62-year-old African man from Burkina Faso with a negative history for urinary tract diseases was admitted to the medical ward of St Jean de Dieu Hospital of Tanguiéta for pericardial effusion associated with dyspnea and lower leg edema. Renal function was normal (serum creatinine: 88 μmol/L) and urinalysis, by dipstick and microscopy, was unremarkable. A diuretic treatment with oral

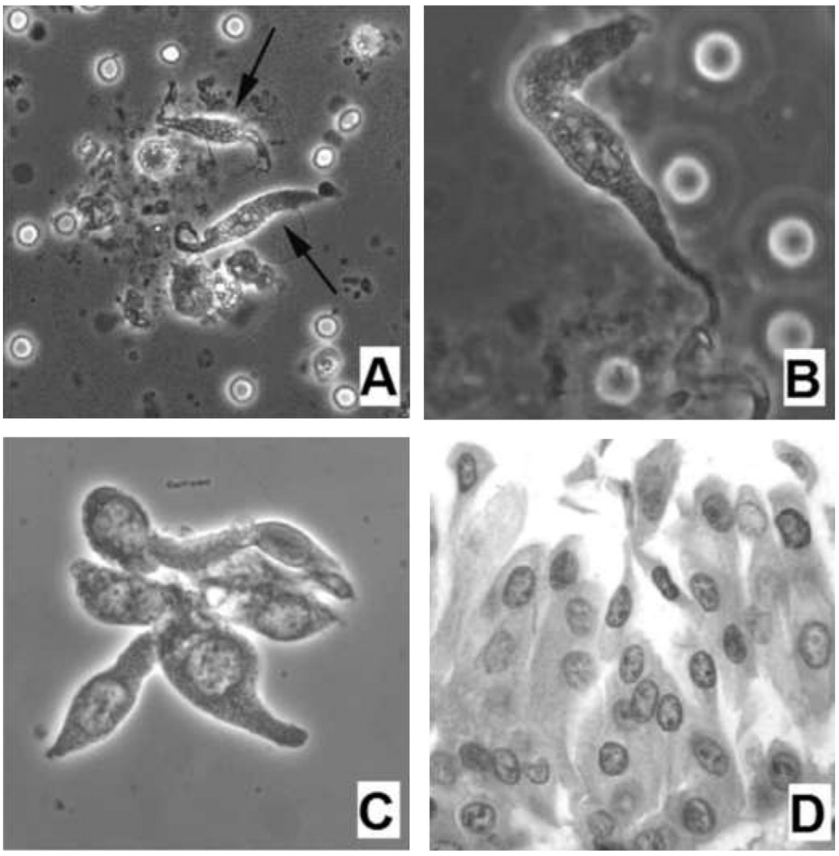

Fig. 1. A: Two spindle cells (arrows) and isomorphic erythrocytes at low magnification (original: 160×). B: A spindle cell (size: 76 × 8.5 μm) at high magnification (original: 800×). C: A cluster of atypical/malignant cells with high N/C ratio (original magnification: 640×). D: Spindle cells in the bladder carcinoma (original magnification: 480×) (A–C: phase contrast microscopy; D: hematoxylin–eosin stain).